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ANNEX 9

Expression of *Erythrina corallodendron* lectin in *Escherichia coli*

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The cDNA of the *Erythrina corallodendron* lectin (*ECor*L) has been expressed in *Escherichia coli*. For this purpose, an *Nco*I site was inserted into the cDNA coding for the lectin precursor [Arango, R., Rozenblatt, S. & Sharon, N. (1990) *FEBS Lett. 264*, 109–112] immediately before the codon GTG (103–105) which codes for the N-terminal valine of the mature lectin. This introduced an ATG codon for a methionine preceding the valine. The mutated cDNA was ligated into pUC-8, then subcloned into the expression vector pET-3d, which carries a strong promoter derived from gene 10 of the phage T7. The recombinant plasmid was introduced into the *E. coli* lysogenic strain BL21(DE3). Recombinant *ECor*L was expressed by growing the bacteria in the presence of isopropyl β-D-thiogalactopyranoside. Most of the recombinant lectin was found in an insoluble aggregated form as inclusion bodies and only a small part was in the culture medium in a soluble active form. Functional recombinant lectin was recovered from the inclusion bodies by solubilization with 6 M urea in cyclohexylaminopropane sulfonate pH 10.5, renaturation by 10-fold dilution in the same buffer and further adjustment of the pH to 8.0. The recombinant lectin, obtained at a yield of 4–7 mg/l culture, had, by gel filtration, a slightly lower molecular mass (56 kDa) than the native lectin, and was devoid of covalently linked carbohydrate; it was, however, essentially indistinguishable from native *ECor*L by other criteria, including its dimeric structure, Western blot analysis with anti-*ECor*L polyclonal and monoclonal antibodies, and Ouchterlony double-diffusion analysis with polyclonal antibodies, as well as hemagglutinating activity and specificity for mono- or disaccharides.

Lectins are carbohydrate-binding proteins present in a variety of organisms ranging from bacteria to higher vertebrates [1, 2]. The most thoroughly studied lectins are those extracted from plants, especially from the seeds of the leguminosae [3, 4]. They serve as invaluable carbohydrate-specific tools in many areas of biological and medical research, and are also used clinically. Legume lectins comprise a large group of proteins that share extensive similarities in their primary amino acid sequences, and possess similar secondary and tertiary structures [4–6]. Despite these similarities, they display a wide range of carbohydrate specificities. The structural basis of these specificities is most likely due to differences in the architecture of the variable parts of their binding sites [7].

Erythrina is a family of over 100 species of deciduous leguminous trees and shrubs found in the tropics and subtropics. Since 1980, lectins from over 20 species of this family have been isolated in different laboratories, ten of these by us [3, 8]. All *Erythrina* lectins are composed of two identical, or nearly identical, subunits with molecular masses close to 30 kDa. They are glycoproteins containing 3–9% carbohydrate and are specific for galactose and *N*-acetylgalactosamine, with a preference for *N*-acetyllactosamine which binds 10–30 times better than galactose [8–10]. Recently, the three-dimensional structure of the complex of *Erythrina corallodendron* lectin (*ECor*L) with lactose has been solved at 0.2-nm resolution [7].

We had previously cloned and sequenced a 1017-bp cDNA fragment containing the entire coding region of *ECor*L [11]. This fragment encodes a polypeptide of 281 amino acids, consisting of a leader sequence of 26 amino acids and a mature lectin of 255 amino acids. We now report the expression and recovery of functional recombinant *ECor*L (r*ECor*L) in *Escherichia coli*. This was accomplished by introducing the region coding for the mature *ECor*L into an expression vector based on the T7 promoter. The availability of this expression system will enable us to perform structure/function relationship studies of the lectin's binding site by site-directed mutagenesis, and also to attempt to modify the specificity of the lectin.

MATERIALS AND METHODS

Materials

Restriction enzymes and DNA-modifying enzymes were obtained from New England Biolabs and United States Biochemical Corporation (Cleveland, OH). DNA sequencing reagents were also obtained from US Biochemical and *Taq* DNA polymerase was obtained from New England Biolabs. *Bam*HI linkers were from Pharmacia; oligonucleotides were from the Chemical Services of the Weizmann Institute of Science; pET-3d was from Novagen (Madison, WI). Native *ECor*L was isolated by affinity chromatography on immobilized galactose

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Abbreviations. Caps, 3-(cyclohexylamino)-1-propanesulfonic acid; *ECor*L, *Erythrina corallodendron* lectin; r*ECor*L, recombinant *ECor*L; IPTG, isopropyl β-D-thiogalactopyranoside; PCR, polymerase chain reaction.

[8, 12] and further purified by affinity chromatography on immobilized concanavalin A as described for *Erythrina cristagalli* lectin [12]. All other chemicals were from commercial sources, of the highest purity available.

Plasmids and fragments

Plasmid DNAs were prepared by the alkali/SDS-lysis procedure and purified with Qiagen-tip20 columns (Diagen GmbH, Düsseldorf) according to the manufacturer's protocols. DNA fragments were purified by extraction from a low-melting agarose gel using standard procedures [13]. Plasmid DNAs were introduced into various strains of *E. coli* by calcium-mediated transformation [13].

DNA manipulations for construction of expression vectors

Digestions with restriction enzymes were performed using buffers and conditions supplied by the manufacturers. When complete digestion was necessary, the reaction mixtures were left overnight. Ligations were done in a reaction mixture containing ligation buffer (50 mM Tris/HCl pH 7.6, 10 mM $MgCl_2$ and 1 mM ATP), 5 units T4 DNA ligase and the DNAs to be ligated at appropriate concentrations [13]; incubation was overnight at 15°C. DNA sequencing was carried out by the dideoxy-chain-termination method [14], using Sequenase version 2.0 kit according to the protocol provided by US Biochemical Corp.

Polymerase chain reaction

The amplification of the *ECor*L mutated fragment was performed in a 100-µl reaction volume containing polymerase chain reaction (PCR) buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), dNTPs (20 µM each), 50 pmol 3′ and 5′ primers, 200 ng plEcl-C (a plasmid containing the *ECor*L cDNA) [11], 2.5 units *Thermus aquaticus* DNA polymerase and two drops of mineral oil. The samples were placed in a thermal controller (MJ Research) programmed for a temperature cycle of 95°C (1 min), 37°C (2 min) and 72°C (2 min). This cycle was repeated 30 times with a 7-min extension at 72°C following the last cycle. The final reaction products were resolved in a 1% agarose gel. The amplified fragment was prepared for subcloning by a fill-in reaction with T4 DNA polymerase.

Expression of *ECor*L

E. coli BL21(DE3), a lysogen containing a single gene of the T7 RNA polymerase under the control of the inducible lacuv5 promotor, was used for expression of *ECor*L [15]. The bacteria with pET-*ECor*L or control pET-3d were grown at 37°C, with shaking, in 10 ml NZYM medium (10 g type A hydrolysate of casein, 5 g NaCl, 5 g yeast extract and 2 g $MgSO_4 \cdot 7\,H_2O$) [13] containing 0.4% glucose and 100 µg/ml ampicillin until mid-log phase (A_{600} of 0.6), then induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) to 0.4 mM and incubation was continued at 25°C or 37°C. Aliquots (1 ml) of bacterial culture were taken after predetermined periods of induction (up to 24 h). The cells were collected by centrifugation and suspended in 80 µl double distilled water and 20 µl SDS/PAGE sample buffer (0.31 M Tris pH 6.8, 10% SDS, 50% glycerol and 0.005% bromophenol blue). Detection of *ECor*L in these bacterial lysates after SDS/PAGE was done by staining with Coomassie brilliant blue R-250 or by Western blot analysis using a rabbit anti-*ECo* antiserum or a murine monoclonal antibody (no. 105) ra against native *ECor*L. The blots were visualized with g anti-rabbit or anti-mouse antibodies coupled to alkaline ph phatase, followed by incubation with nitro-blue tetrazoli (0.3 mg/ml) and bromochloroindolyl phosphate (0.15 mg/ in *N*,*N*-dimethylformamide [16]. To obtain larger amounts r*ECor*L, the *E. coli* BL21(DE3) cells containing pET-*ECor* were grown as for small-scale expression except that 1 l m dium was used and, after addition of IPTG to a final conc tration of 0.4 mM, the temperature was shifted to 25°C a the culture was further incubated overnight at the same te perature.

Preparation of inclusion bodies

Cells from an overnight 1-l culture were collected b centrifugation, suspended in 100 ml 0.15 M NaCl, 50 m Tris/HCl pH 8 (NaCl/Tris buffer), containing 0.3 mg/ lysozyme, and left overnight at −20°C. Lysis of the bacteri cells was done by thawing, adding 0.5 ml 1 M $MgCl_2$ a DNase I to a final concentration of 7 µg/ml and sonicati for 5 min with a Branson B-12 sonicator. The insoluble m terial was then collected by centrifugation at 8000 × *g* in Sorvall RC-5B centrifuge, and washed twice in 1 l NaCl/Tr buffer with 1% Triton X-100 in order to solubilize and remov membranes and membrane-bound proteins. The washing were done by stirring the insoluble material with a magnetic stirrer for 1 h at room temperature followed by centrifugation as above. The insoluble material obtained after the last wash ing was kept at −20°C until used for solubilization.

Solubilization and refolding

r*ECor*L inclusion bodies were solubilized with 10 ml 6 M urea in 10 mM 3-(cyclohexylamino)-1-propanesulfonate (Caps) pH 10.5. The volume of the buffer-containing urea was increased until the absorbance of the solution at 280 nm was below 0.5. Refolding was done by slowly diluting the urea solution tenfold with 10 mM Caps pH 10.5, followed by addition of 0.01 vol. 1 M Tris pH 8, and further adjustment of the pH to 7.5 using concentrated HCl. The resulting solution was then concentrated by ultrafiltration to about 50 ml using a Millipore (Bedford, MA) Minitan acrylic ultrafiltration system and was dialyzed extensively against 10 mM Tris/HCl pH 7.5, containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. A precipitate of the non-refolded lectin, which was always formed during this procedure, was removed by centrifugation (8000 × *g*, 15 min in the cold) before storage of the lectin solution at −20°C. Prior to any characterization test, the lectin solution was further concentrated (using an Amicon ultrafiltration stirred cell with a PM-10 membrane) to approximately 1 mg/ml, estimated by absorbance at 280 nm on the assumption that a solution containing 1 mg/ml of *ECor*L has the same absorbance as *E. cristagalli* lectin, namely 1.53 [9].

SDS/PAGE and detection of proteins in gels

Denaturing PAGE was performed according to Laemmli [17] using 12% separation gels and 2.5% stacking gels.

Analytical molecular sieve chromatography

The molecular masses of the native and recombinant *ECor*L were determined by gel filtration using a Sephadex G-

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column (1.5 × 90 cm) equilibrated with 10 mM Tris/HCl pH 7.5, 0.15 M NaCl; elution was with the same buffer. The column was calibrated with the following proteins of known molecular mass: bovine serum albumin (67 kDa), ovalbumin (43 kDa), bovine chymotrypsinogen (25 kDa) and bovine ribonuclease A (13.7 kDa).

Gel diffusion

Gel diffusion was done according to Ouchterlony [18] except that 1% Triton X-100 was added to 1% agarose in phosphate-buffered saline [15 mM, pH 7.2].

Hemagglutinating activity

The hemagglutinating activity of the lectin was assayed by the serial dilution method in microtiter plates [19] except that 25 µl lectin solution and 25 µl 4% suspension of human-type O erythrocytes were used. A unit of activity is defined as the lowest concentration of lectin giving visible agglutination. The inhibitory activity of sugars was measured by mixing serial dilutions of the sugar with 4 hemagglutinating units of the lectin before the addition of erythrocytes and determining the lowest concentration giving full inhibition of agglutination.

N-Terminal sequence determination

Native and recombinant *ECor*L (50 µg) were run on a 12% SDS gel, and electrophoretically transferred to a polyvinylidene difluoride membrane. The membrane was stained with 0.1% Coomassie brilliant blue R250, bands corresponding to the lectin were cut out from the membrane [20] and sequenced on a gas-phase Applied Biosystem automatic sequencer, model 470A.

Carbohydrate determination

Total neutral carbohydrate content was determined by the phenol/H_2SO_4 method [21] using mannose as reference sugar.

RESULTS

Construction of the expression vector pET-*ECor*L

For expression of *ECor*L in *E. coli*, the vector pET-3d was used [15, 22]. This plasmid is a derivative of pBR322 with a strong promoter, an efficient ribosome binding site and a translation initiation region all derived from gene 10 of phage T7 (which codes for a major capsid protein of the phage). The initiation ATG codon of gene 10 is located in a unique *Nco*I site and a unique *Bam*HI site is located upstream of gene 10 transcription termination region [15]. In order to introduce the cDNA coding sequence of the complete mature *ECor*L [11], an *Nco*I site was created immediately before codon GTG at position 103–105 which, in this sequence, codes for Val27, the first amino acid of the mature lectin [23]. This *Nco*I site also introduces an ATG preceding Val27 and will code for N-terminal methionine in the recombinant lectin.

Fig. 1 shows the creation of the *Nco*I site using PCR with a 5′ end primer (CAAAGTTAACCATGGTGA) at positions 90–107 containing the necessary mutations (bold letters) and another primer (ACCATTGTTGCAGGTGT) at positions 916–932 of the 3′ end of the *ECor*L cDNA; plEcl-C [11] was the target DNA of the PCR reaction. A fragment of 800 bp

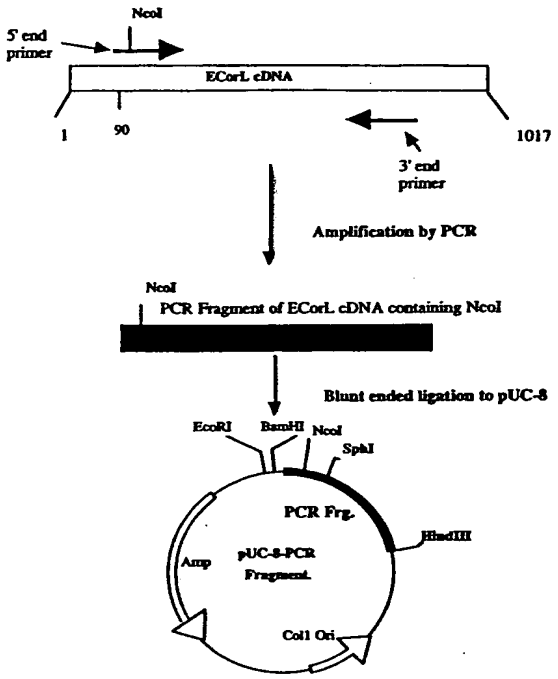

Fig. 1. Creation of *Nco*I site on *ECor*L cDNA with PCR.

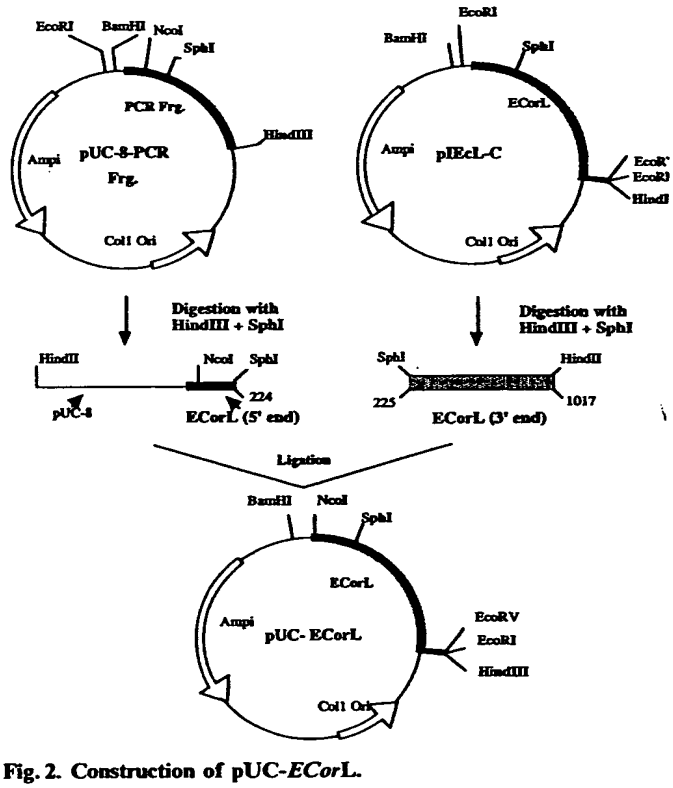

Fig. 2. Construction of pUC-*ECor*L.

was obtained and subsequently subcloned in a blunt-end fashion into a pUC-8 vector previously digested wtih *Hinc*

In order to reconstruct the entire coding sequence of t lectin, an internal *Sph*I site, located at position 224 of t nucleotide sequence, was used as shown in Fig. 2. The pU

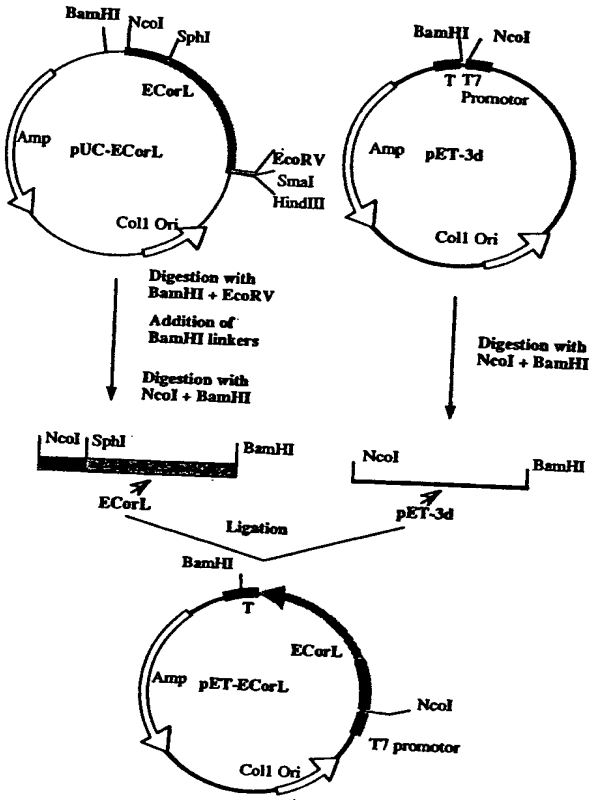

Fig. 3. Construction of pET-*ECorL*.

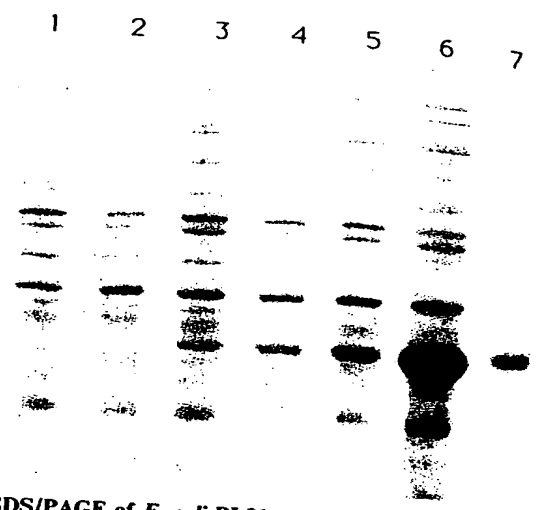

Fig. 4. SDS/PAGE of *E. coli* BL21 carrying pET-*ECorL*. Cells were grown at 37°C until late-log phase and then induced by the addition of IPTG to 0.4 mM. At different time points, 1-ml aliquots of the culture were taken, cells collected by centrifugation and suspended in 100 µl SDS/PAGE sample buffer. SDS/PAGE was performed by the Laemmli procedure [17] with 12% gels. Proteins were visualized by Coomassie brilliant blue. Lane 1, BL21 cells with pET-3d alone; lane 2, cells containing pET-*ECorL* before the addition of IPTG; lanes 3–6, cells containing pET-*ECorL* induced with IPTG for 1, 2, 3 and 18 h, respectively; lane 7, *ECorL*.

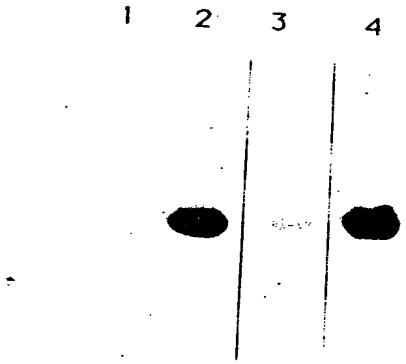

Fig. 5. Western blot analysis of *E. coli* BL21 cells carrying pET-*ECorL*. Crude lysates from bacteria carrying pET-*ECorL* were size fractionated on a 12% SDS/PAGE and transferred to a nitrocellulose membrane. Blots were probed with polyclonal rabbit anti-*ECorL* antiserum or with a mouse monoclonal antibody raised against the lectin. Lane 1, BL21 cells with pET-3d alone probed with polyclonal antiserum; lanes 2 and 3, cells containing pET-*ECorL* induced with IPTG for 3 h at 37°C, probed with polyclonal antiserum and a monoclonal antibody, respectively; lane 4, native *ECorL* probed with polyclonal antiserum.

8 vector containing the PCR fragment was digested with *Sph*I and *Hin*dIII, generating two fragments, the first containing the vector sequences plus 124 bp of the 5′ end of *ECorL* cDNA which contains the *Nco*I mutation and the other (not shown in Fig. 2), a *Sph*I–*Hin*dIII fragment of 641 bp containing most of the 3′ coding sequence of the lectin. plEcl-C was digested with *Sph*I and *Hin*dIII releasing a fragment that contains all the coding sequence of *ECorL* at the 3′ end of the *Sph*I site (nucleotides 225–868) and a 3′ nontranslated sequence (nucleotides 869–1017); the first fragment obtained from the pUC-8-PCR vector and the 3′ end fragment from the plEcl-C vector were ligated, creating a pUC-8 plasmid, designated as pUC-*ECorL*, which contained the entire coding sequence of the mature *ECorL* with the *Nco*I mutation before the N-terminal valine of the mature lectin. Sequence determination of the 5′ end of this construct, up to the *Sph*I site, confirmed the *Nco*I mutation and ensured that no other undesired mutations had been produced.

The *ECorL* cDNA containing the *Nco*I site was then subcloned into the expression vector pET-3d as shown in Fig. 3. pUC-*ECorL* was digested with *Bam*HI and *Eco*RV, releasing the entire *ECorL* coding fragment and the non-coding sequences. *Bam*HI linkers were ligated to the blunt-ended *Eco*RV end of the fragment. This linked fragment was then digested with *Nco*I and *Bam*HI and the product was ligated to the pET-3d plasmid previously opened by digestion with *Nco*I and *Bam*HI, to generate a recombinant plasmid designated as pET-*ECorL*.

Expression of *ECorL* in *E. coli*

Fig. 4 shows the results of SDS/PAGE analysis of lysates of *E. coli* BL21(DE3), containing pET-*ECorL*, before and after induction with IPTG at 37°C. For comparison, analysis of a lysate of the parent strain containing pET-3d is also included. A band that migrates slightly faster than the native lectin is visible in the lysates after 1 h of induction. Western blot analysis of the cell lysates revealed that this protein band reacts with rabbit anti-*ECorL* antiserum as well as with a murine monoclonal antibody raised against the native lectin (Fig. 5). The amount of r*ECorL* increased (up to 150 mg protein/l culture) when the temperature during the induction was lowered from 37°C to 25°C (Fig. 6).

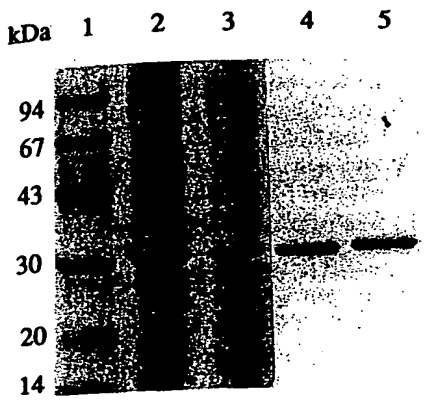

Fig. 6. Isolation of r*ECor*L from *E. coli*. Protein samples were analyzed by electrophoresis on 12% SDS/PAGE and stained with Coomassie blue. Lane 1, molecular mass markers: phosphorylase *b* 94 kDa, albumin 67 kDa, ovalbumin 43 kDa, carbonic anhydrase 30 kDa, trypsin inhibitor 20.1 kDa, α-lactalbumin 14.4 kDa. Lanes 2 and 3, bacterial lysates of BL21 cells induced with IPTG overnight at 25°C and 37°C, respectively; lane 4, recombinant *ECor*L purified from inclusion bodies; lane 5, native *ECor*L.

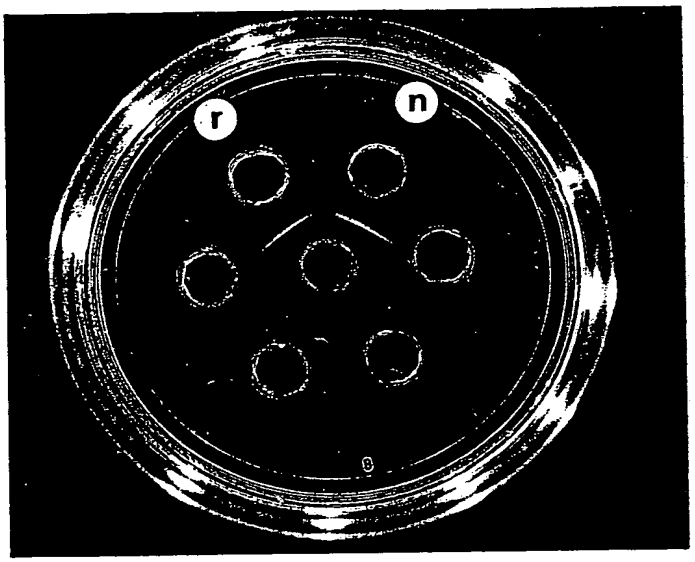

Fig. 7. Ouchterlony double-diffusion analysis of recombinant and native *ECor*L with anti-*ECor*L polyclonal antibodies. Wells: r, r*ECor*L, 10 μg; n, *ECor*L, 10 μg; center, 10 μl undiluted rabbit anti-*ECor*L antiserum.

Purification and refolding of recombinant *ECor*L

A small amount of soluble r*ECor*L was found in the culture medium and isolated by immunoaffinity chromatography on an anti-*ECor*L column. However, the yield was so low (less than 0.1 mg/l) that it was not studied in detail. The insoluble fraction of the cell lysate contained most of the r*ECor*L in the form of inclusion bodies and was recovered by centrifugation. Contaminating membrane-bound proteins, fragmented cell walls and membranes were removed by several washings in a buffer containing Triton X-100 and lysozyme. This procedure yielded a pellet of protein aggregates, which, upon analysis by SDS/PAGE, proved to contain almost exclusively the recombinant lectin (data not shown). Soluble, active protein was recovered by dissolving the inclusion bodies in a large volume of 6 M urea, and removal of the denaturant under alkaline conditions (pH 10.5) as described under Methods. After the refolding step, the protein preparation was analyzed by SDS/PAGE and proved to contain only the recombinant lectin (Fig. 6). Typically, 4–7 mg active r*ECor*L from 1 l culture was obtained, which represented a yield of approximately 3–4% of the total r*ECor*L present in inclusion bodies.

Characterization of r*ECor*L

SDS/PAGE

As shown in Fig. 6, r*ECor*L has an apparent subunit molecular mass of close to 28 kDa on SDS/PAGE, slightly lower than the native protein. This is consistent with the absence in the r*ECor*L of the carbohydrate moiety present in *ECor*L (one heptasaccharide, molecular mass 1171 Da/subunit [7, 24]). Indeed, analysis for neutral sugar confirmed that the recombinant lectin contains less than 0.7% carbohydrate, compared to about 6% in native *ECor*L.

Molecular mass determination

The molecular mass of the recombinant protein, as estimated by gel filtration, was around 56 kDa, compared to 60 kDa of the native protein, showing that both proteins form dimers in solution. The slightly lower molecular mass of the recombinant protein can be accounted for by the absence of covalently bound carbohydrate.

Immunological assays

Blots of the purified recombinant lectin reacted with the rabbit anti-*ECor*L antibodies as well as with six monoclonal antibodies prepared against the native *ECor*L (data not shown). In addition, on Ouchterlony gel immunodiffusion analysis using polyclonal antibodies to the native *ECor*L, the recombinant and the native lectin developed lines of identity with each other (Fig. 7).

N-terminal sequence

In r*ECor*L 70% of the lectin molecules have the N-terminal sequence Met-Val-Glu-Thr-Ile, whereas 30% have Val-Glu-Thr-Ile-Ser, which is the same as the N-terminal pentapeptide of the native lectin [23].

Hemagglutination assays

The minimal hemagglutinating concentration of r*ECor*L was the same as that of the native protein (3 μg/ml and 3.3 μg/ml, respectively). The results of inhibition of hemagglutination are given in Fig. 8. The hemagglutinating activity of both lectins was 2–4 times more sensitive to inhibition by *N*-acetylgalactosamine than by galactose. Methyl β-*N*-dansyl-galactosaminide was a powerful inhibitor, being 256 and 512 times stronger than galactose for *ECor*L and r*ECor*L, respectively. The two lectins were equally sensitive to *N*-acetyllactosamine (16 times more than to galactose) and no inhibition was observed by 250 mM glucose or mannose.

DISCUSSION

In this paper, we describe the construction of an expression vector containing the entire coding sequence of the mature

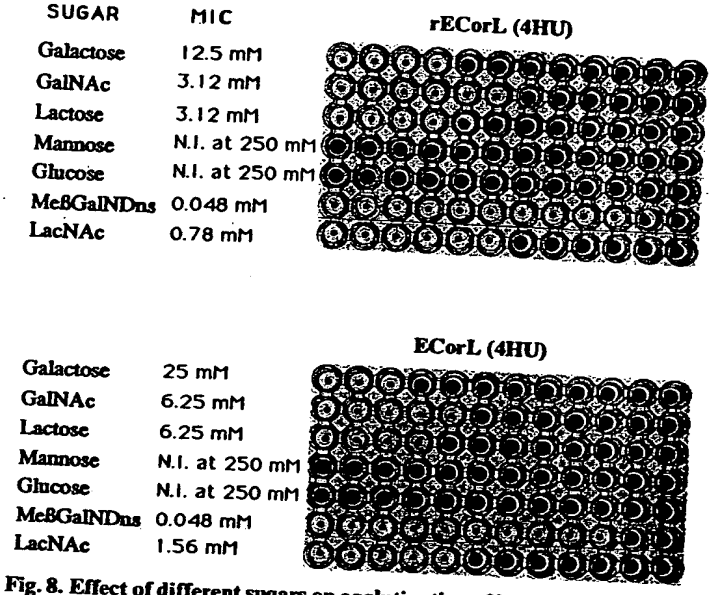

Fig. 8. Effect of different sugars on agglutination of human erythrocytes by native and recombinant *ECor*L. Each well contains 4 hemagglutinating units of the lectin, plus serial double dilutions of the inhibitor. The sugar used and the minimal concentration at which it is inhibitory (MIC) are indicated on the left. MeβGalNDns, methyl β-*N*-dansylgalactosaminide; LacNAc, *N*-acetyllactosamine; N.I., not inhibitory.

*ECor*L, its expression in *E. coli*, and the isolation and characterization of a functionally active recombinant lectin. Although small quantities of the lectin were found in soluble active form in the culture medium, the bulk was in the form of inclusion bodies from which active protein could be recovered after solubilization with urea and refolding in alkaline conditions. Accumulation of recombinant proteins as insoluble aggregates in *E. coli* is a common phenomenon [25]. To obtain an active protein from these aggregates usually necessitates the use of strong denaturing agents, such as urea or guanidinium hydrochloride, followed by refolding of the protein to its native conformation [25]. This last step requires somewhat different conditions, which are unique to each protein and have to be determined empirically. In our case, the alkaline pH of the buffer used for diluting out the urea, and a concentration of protein of less than 0.35 mg/ml ($A_{280} < 0.5$) before dilution, were critical for the recovery of active protein; when neutral buffers or higher protein concentrations were used, all the recombinant protein precipitated immediately.

Several legume lectins have been successfully expressed in *E. coli* [26–28]. Recombinant pea lectin was found to be expressed in the form of inclusion bodies, which were solubilized with guanidinium hydrochloride but, in contrast to our case, the refolding step did not require the use of alkaline conditions and the yield of active protein was higher than ours (between 10–20%) [26].

The recombinant *ECor*L is very similar in its properties to the native protein as shown by a number of criteria: gel-filtration chromatography showed that the recombinant lectin exists in solution as a dimer; Ouchterlony double-diffusion analysis with polyclonal antibodies and Western blot analysis with several monoclonal antibodies showed that both lectins are immunologically indistinguishable. There are, however, minor differences between recombinant *ECor*L and the native protein: r*ECor*L is not glycosylated and, as a result, its molecular mass is slightly lower than that of the native lectin. A 70% of r*ECor*L molecules contain an additional methio at the N-terminal. Our results indicate that these differe do not affect the hemagglutinating activity of the protein its carbohydrate specificity. Indeed, both the native and recombinant lectins are inhibited by the same spectrum carbohydrates without any significant differences, eit qualitatively or quantitatively. Taking into consideration low accuracy of the hemagglutination assay, our data ar good agreement with the relative inhibitory activities repor for *ECor*L [8, 10, 29] and in the range found for ot *Erythrina* lectins [3, 8]. This point is of crucial importance further structure/function relationship studies on the lecti carbohydrate binding site. The finding that the carbohydr moiety of *ECor*L has no effect on its binding properties s ports other reports showing that carbohydrate-free combinant lectins are as active as the native ones [28]. T the activity of glycoprotein lectins is independent of th covalently bound carbohydrate was proposed by us some years ago [30].

We now have a system that allows us to alter individ amino acids in the binding site of *ECor*L and in this w to study their effect on the sugar specificity of the prote According to currently proposed models, based on X-ray cr tallography of complexes of sugars with *ECor*L and oth lectins [7, 31–34], the ligand-binding specificity displa by these proteins is attained by the coupling of adjustab topological parameters, such as the outline of the β-turns a shape of the variable residues lining the binding pocket, wi a conserved constellation of residues (Asp89, Gly107, Phe1 and Asn133 in *ECor*L) involved directly in hydrogen bon and hydrophobic interactions with the bound sugar. Alte ation of selected amino acid residues and sequences withi the carbohydrate-binding pocket of r*ECor*L, by site-directe mutagenesis, will provide us with valuable information for better understanding of the carbohydrate specificity of legum lectins, and hopefully of lectins from other sources as well.

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